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Onchocerca volvulus: the road from basic biology to a vaccine

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New York Blood Center
Lindsley F. Kimball Research Institute

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Editors, *Trends in Parasitology*

Dear Editor,

Many thanks for inviting us to contribute to *Trends in Parasitology*.

We submit for your consideration the revised invited review manuscript entitled: "***Onchocerca volvulus***: the road from basic biology to a vaccine**".**

Thank you for the thorough and helpful comments from yourself and the reviewers who looked at our manuscript. We have now addressed all of their concerns, leading to a significantly revised and improved review manuscript. A detailed response to both your editorial comments and those of the reviewers are included below – in almost every case we have followed the reviewers' recommendations. In the tracked revision, the edits are marked in red.

We hope you feel this paper is now acceptable for publication in *Trends in parasitology* and look forward to hearing from you,

Sincerely,

The corresponding author, on behalf of all authors

Sara Lustigman (slustigman@nybloodcenter.org)

Onchocerca volvulus: the road from basic biology to a vaccine

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Abstract

Human onchocerciasis—commonly known as river blindness—is one of the most devastating yet neglected tropical diseases, leaving many millions in Sub-Saharan Africa blind and/or with chronic disabilities. Attempts to eliminate onchocerciasis, primarily through the mass drug administration of ivermectin remains challenging and has been heightened by the recent news that drug-resistant parasites are developing in some populations after years of drug treatment. Needed, and needed now, in the fight to eliminate onchocerciasis are new tools, such as preventive and therapeutic vaccines. This review summarizes the progress made to advance the onchocerciasis vaccine from the research lab into the clinic.

Why a vaccine against Onchocerca volvulus is needed

Human onchocerciasis caused by *Onchocerca volvulus* and spread by the bite of infected *Simulium* black flies remains one of the most important neglected tropical diseases (NTDs). Recent estimates from the Global Burden of Disease Study 2015 indicate that approximately 15.5 million people currently live with onchocerciasis, including 12.2 million people with *Onchocerca* skin disease (OSD) and 1.025 million with vision loss (river blindness) [1]. Almost everyone severely affected with OSD and river blindness lives in Sub-Saharan Africa or Yemen in the Middle East.

Through programs of mass drug administration (MDA) with ivermectin, tremendous strides have been made in reducing the global prevalence of onchocerciasis. Transmission has been nearly eliminated in Latin America, while globally there has been a 29 percent reduction in the prevalence of onchocerciasis since 2005 [1]. However, it remains unlikely that onchocerciasis can be eliminated as a public health problem entirely through ivermectin mass treatments. The reasons for this observation have been reviewed recently, and include the inability to implement large-scale treatment programs in areas that are co-endemic for loiasis, and the potential for emerging anthelminthic drug resistance [2]. Recent genome-wide analyses revealed genetic variation that significantly differentiated *O. volvulus* parasites that are good responders to treatment with ivermectin to *O. volvulus* parasites that are sub-optimal responder and taken from individuals in Ghana and Cameroon that have experienced

repopulation of the skin microfilariae earlier/more extensively after ivermectin treatment than expected [3].

In addition, disease modeling studies show that transmission interruption and elimination will require routine and regular quantum reductions in *O. volvulus* microfilariae in the skin and subcutaneous tissues following each round of MDA, but such targets are seldom achieved [2]. The African Programme for Onchocerciasis Control predicted in 2015 that to achieve elimination 1.15 billion treatments will have needed to be administered until 2045 [4]. Such estimates indicate that onchocerciasis may not be eliminated for decades using current approaches.

To accelerate elimination and advance towards the major targets of the 2012 London Declaration for NTDs (http://unitingtocombatntds.org/sites/default/files/document/london_declaration_on_ntds.pdf), there is an effort to develop new and improved control tools. These include better diagnostics, small-molecule drugs and vaccines that can improve surveillance and achieve longer and more sustained reductions in host microfilarial loads. There is also a need for better safety profiles for interventions used in loiasis co-endemic areas of Africa. Individuals who have high blood levels of *Loa loa* microfilariae, a filarial infection that usually does not cause clinical disease, and receive ivermectin as part of the MDA programs to eradicate lymphatic filariasis and onchocerciasis may develop a severe inflammatory reaction that can result in encephalopathy, and rarely death. In 2015, an international consortium launched a new global initiative, known as TOVA – The

Onchocerciasis Vaccine for Africa [2]. TOVA is evaluating and pursuing vaccine development as a complementary control tool. Briefly, TOVA is primarily using recombinant proteins and novel adjuvant platforms, with the goal to meet at least one of the desired target product profiles (TPP). The TPP either relies on a preventive vaccine for children under the age of five who have not yet had access to MDA with ivermectin, or a therapeutic vaccine for both adults and children with onchocerciasis (Table 1) [2]. The efforts to develop an effective, safe, and logistically feasible vaccine against onchocerciasis builds on the evidence of protective immunity achieved using live attenuated vaccines. Immunization with irradiated larvae typically achieves ~70% protection in laboratory settings [5-9], but such vaccines are not feasible for mass human immunization on safety, logistical, and economic grounds. Current efforts to develop a subunit vaccine, such as confirmatory vaccine trials in large-animal models, modeling studies, and future clinical trials will build the necessary body of evidence to allow for the selection of the best TPP. The TPP presented in Table 1 was based in part on mathematical modelling that explored the potential influence of a prophylactic vaccination program on infection resurgence in areas where local elimination has been successfully achieved [10]. It assumed based on efficacy results in animal models of an initial prophylactic efficacy of 50%, and an initial therapeutic efficacy of 90%. The vaccine was assumed to target 1 to 5 year olds based on the age range included in the Expanded Programme on Immunization. The modelling indicated that an onchocerciasis vaccine would have a beneficial impact in onchocerciasis-loiasis co-endemic areas, markedly reducing microfilarial load in the young (under 20 yr) age groups. The TPP for therapeutic vaccines is still hypothetical as it assumes that it will be safe to target

111 immunologically residual microfilariae in young and adult population living in endemic
112 regions that went through many years of MDA with ivermectin.
113
114 Here, we provide a perspective of the importance of a rational design for the discovery
115 and antigen selection process before embarking into advanced vaccine development of
116 the onchocerciasis vaccine with a review of the current advancements and progress on
117 the TOVA global initiative. Finally, we provide a prospective of how new technologies
118 and artificial intelligence can catalyze and accelerate the evaluation and selection of
119 suitable vaccine candidates leading to a greater chance of their translation into safe and
120 efficacious human vaccines.
121
122 **Discovery and evaluation of the first generation vaccine candidate antigens**
123
124 Considerable effort has been expended in the 1990s on the identification of parasite
125 molecules, primarily proteins, which induce a protective immune response in humans
126 and in the available animal models of onchocerciasis. Anti-L3 protective immunity within
127 the *O. volvulus* endemic population have been described in two populations: (1)
128 immunity that impedes the development of a patent infection (microfilaria positive) in the
129 putatively immune (PI) individuals (i.e., individuals that had no clinical manifestations of
130 the disease, even though they lived for at least 10 years within regions where
131 onchocerciasis is endemic and were exposed to high transmission rates of infection);
132 and (2) concomitant immunity that develops in the patently infected individuals with
133 increasing age and which is independent of the immune responses that are induced by

the adult worms and microfilaria associated with patent infection [11]. Protective immunity against the infective larvae was also shown in a mouse model employing *O. volvulus* L3 in diffusion chambers; a significant reduction of ~50% in the survival of larvae was obtained in mice immunized with normal, irradiated or freeze-thaw-killed L3 [5].

Two basic strategies were used to identify and clone *O. volvulus* target vaccine antigens: **(1)** Exploitation of the potential involvement of antibodies in protective immunity by immunoscreening various *O. volvulus* cDNA libraries to identify target proteins. The success of the immunoscreening effort relied mostly on the source and specificity of the immune sera from human or animal hosts and, hence, was done mostly with serum samples from individuals identified as putatively immune. In addition, sera from vaccinated or immune animals (chimpanzees, mice or cows), polyclonal antibodies raised against *O. volvulus* infective stage larvae also called L3, or monoclonal antibodies developed against specific parasite-antigens, were used to screen the cDNA libraries. Initially cDNA libraries constructed from adult worm stages of *O. volvulus* were used and later cDNA libraries constructed from *O. volvulus* larval stages (L3, molting L3 and fourth-stage larvae or L4) were used. Altogether, out of 26 recombinant antigens that were identified by immunoscreening and tested in the *O. volvulus* mouse model, 12 induced partial but significant protection (39–69%) in the presence of block copolymer, alum or Freund's complete adjuvant [11-13]. **(2)** Identification and isolation of molecules thought to be essential during the infection process. These molecules would include proteins with vital metabolic functions or

defense properties, which would permit the parasite to survive in immunocompetent hosts. Targeting such molecules as vaccine candidates, would block or interfere with the establishment of the parasite in the host. In addition, antigens that are not normally seen by the host, but that are nevertheless accessible to host immune-effector molecules and cells, the 'hidden antigens', were also thought to be potentially useful as vaccine targets [14]. The identification of the genes and isolation of the encoding proteins of interest was achieved by one or multiple of the following methods: a) screening cDNA libraries using a heterologous probes [15]; b) amplification by PCR using degenerate primers and cloning strategies [15]; c) purification of the proteins from secreted products of larval stages followed by partial amino acid sequencing and molecular cloning [16]; or d) identification of the genes of interest by searching the *O. volvulus* expressed sequence tag (EST) database or the EST databases generated by the Filarial Genome Project [17]. Out of 18 recombinant antigens that have been cloned using these strategies and that were tested in the *O. volvulus* mouse model, four (*Ov*-ALT-1, *Ov*-CHI-1, *Av*-ABC and *Av*-UBI) induced partial but significant protection. Of these, *Av*-ABC and *Av*-UBI were cloned from the rodent filarial parasite *Acanthocheilonema viteae* and were protective in the presence of alum or Freund's complete adjuvant, as was *Ov*-ALT-1. In addition, chitinase, *Ov*-CHI-1, effectively induced protection using DNA immunization [18]. The *Onchocerca* homologue of *Av*-ABC has not been studied yet, whereas the *Av*-UBI of *A. viteae* is completely identical to *Ov*-UBI.

The characteristics of the parasite proteins corresponding to the above protective recombinant *O. volvulus* antigens have been described in detail previously [12, 13, 19]. Eight of the proteins, *Ov*-ALT-1, *Ov*-B8, *Ov*-RAL-2, *Ov*-B20, OI5/OI3, *Ov*-CHI-1, *Ov*-RBP-1 and *Ov*-103 are parasite specific antigens, whereas *Ov*-ASP-1 is a member of the vespid venom allergen-like protein family [20]. Six of the protective proteins are homologues to recognized proteins of higher organisms. Thus, *Ov*-CPI-2 (onchocystatin), *Ov*-TMY-1 (tropomyosin), *Ov*-FBA-1 (aldolase), *Ov*-CAL-1 (calponin), *Av*-ABC (ATP binding cassette protein transporter) and *Av*-UBI (ubiquitin) have 32, 31, 69, 42, 71 and 98% amino-acid identity, respectively, with human proteins. An important concern associated with vaccine antigens belonging to conserved gene families (e.g. enzymes, muscle proteins) is the risk of cross-reactions with host or environmental antigens. Eight antigens were also cloned from a very close relative of *O. volvulus*, *O. ochengi*, and used together to vaccinate cattle in the only field trial of a recombinant onchocerciasis vaccine performed to date [21]. These eight antigens included representatives from the parasite-specific [*Oo*-ALT-1, *Oo*-B8, *Oo*-RAL-2, *Oo*-B20 and Oo-FAR-1 (homolog of *Ov*-RBP-1)] as well as the highly conserved (*Oo*-TMY-1 *Oo*-FBA-1, and *Oo*-CPI-2) protein groups. The multivalent vaccine induced statistically significant protection also against patency (microfilaridermia), but did not significantly reduce adult worm burden [22].

Since the above described studies, only one additional antigen with protective properties, *Ov*-GAPDH, which was cloned using immunoscreening, has been recently reported [23]. Thus out of a total of 16 vaccine candidates, 12 were identified by

immunoscreening and 4 were identified using other approaches as illustrated in Figure 1. Below we will describe the 8 vaccine candidates chosen to be studied more in depth for their ability to insure protection against infection.

Evaluation and selection of the best vaccine candidates for a prophylactic vaccine using two small animal models

Humans are the only definitive hosts of *O. volvulus*. Therefore, one of the significant challenges towards the development of a vaccine against onchocerciasis has been the absence of suitable small animal models that support the life-cycle of the parasite (Fig. S1). To overcome this obstacle, we adopted a dual-model screening system. In the first model, *O. volvulus* L3 within diffusion chambers constructed from 14 mm Lucite rings covered with 5.0 µM pore-size membranes are implanted in a subcutaneous pocket on the rear flank of mice [24]. This model has the advantages of using the target human parasite and allows the unique analysis of the host molecules and cells found within the parasite microenvironment. In addition, dissection of the mechanism of immunity induced by the vaccine can be accomplished with the plethora of reagents and assays designed for murine studies. A significant disadvantage of the mouse diffusion chamber model is that the parasites will only develop for a limited time in mice and thus adult worms and microfilariae do not develop. To overcome this limitation, we tested in parallel a second system, the *Brugia malayi*-gerbil model of lymphatic filariasis, using homologues of promising *O. volvulus* antigens. Injection of L3 subcutaneously in this

model allows for examination of vaccine efficacy following the natural migration of developing stages of parasites and their maturation to adult stages [25].

From the pipeline of potential candidate antigens (Fig. 1), fifteen proteins were evaluated in previous studies using the mouse-*Onchocerca* model and identified as being able to induce partial protection following vaccination [13]. To select the most promising protective antigens for the early pre-clinical process development a scoring system was developed that allowed ranking these 15 antigens based on their other known characteristics (reviewed in ref [13]), and to select eight vaccinate candidate for more extensive studies. All the 15 *O. volvulus* protective antigens in the *O. volvulus* - mouse model were given a score of 1.0 (Supplement Table 1). The added scoring was based on the following criteria: (1) score 0.2 was given to those that are nematode or parasite specific with or without known function (for example *Ov*-CPI-2 (cystatin), *Ov*-RBP-1 (retinoid binding protein) or *Ov*-CHI-1 (chitinase); (2) score 0.2 was given to those in which localization of the corresponding native proteins in L3 and/or mL3 by immunoelectron microscopy was in one or more regions that are also recognized by antibodies from protected humans and/or also from xL3 immunized and protected mice [11]; (3) score 0.2 was given to those being recognized by antibodies from protected humans (PI and INF with concomitant immunity) and/or animal models after immunization with xL3 (cattle, chimpanzees, mice); (4) score 0.2 was given to those being abundantly expressed in L3 and/or mL3, which indirectly indicates that the corresponding translated proteins are important for the parasite during the initial phases

of the *Ov* infection; and (5) score 0.2 was given to those where studies have shown the ability of antibodies targeting the parasite antigen to kill larvae *in vitro*.

In addition, we have added two more criteria that are based on more recent published and unpublished studies and thus provide added support for the selection of these 8 antigens for our proposed preclinical studies. A score of 1.0 was given to those (for examples *Ov*-ALT-1, *Ov*-CPI-2, *Ov*-RAL-2, chitinase, *Ov*-RBP-1 and *Ov*-B20) whose homologues have been shown to also induce protection in other filariae host–parasite systems [26-36]. Moreover, A score of 1.0 was given to those (*Ov*-ASP-1, *Ov*-103, *Ov*-CPI-2 , *Ov*-RAL-2) having homologues in other nematode host–parasite systems that have been shown to be able to induce reduction in worm burden or other protective measures against hookworm infection in dogs and *Ascaris* in pigs [37-44]. Based on this rational innovative scoring system we have selected the top ranking 8 *Ov* protective antigens (*Ov*-CPI-2, *Ov*-ALT-1, *Ov*-RAL-2, *Ov*-ASP-1, *Ov*-103, *Ov*-RBP-1, *Ov*-CHI-1 and *Ov*-B20) for which we propose to conduct extensive preclinical evaluation and further selection. Those selected are ranked between a total score of 4.0 to 2.6 (Supplement Table 1). Those of the original 15 r*Ov*Ags that were not selected were only ranked at a total score of 1.0 to 1.6.

The eight selected *O. volvulus* proteins and the *B. malayi* homologues were expressed in both bacterial (*Escherichia coli*) and eukaryotic (*Pichia*) expression systems. In the presence of the adjuvant alum, the recombinant *Ov*-103 and *Ov*-RAL-2 proteins, together with their *Bm*-103 and *Bm*-RAL-2 homologues emerged as the most promising

candidates in each animal model, validating the robustness of our selection and prioritization process. Combination of these two antigens by either co-administration vaccine strategies or single injections using a recombinant fusion protein vaccine induced enhanced levels of protective immunity, demonstrating that the antigens could act synergistically in both systems [45, 46]. Furthermore, these co-administered molecules or the fusion proteins reduced embryogenesis in *B. malayi* females, suggesting a potential impact also on microfilaremia and transmission [46].

Various adjuvants were evaluated and compared for their ability to improve efficacy by enhancing the killing of *O. volvulus* in diffusion chambers implanted in mice. Only adjuvants that induced Th2 responses, as determined by cytokine profiles, were effective at enhancing the vaccine efficacy, consistent with reports showing that IL-4, IL-5, and functional eosinophils are necessary for the development of adaptive immunity in mice immunized with irradiated *O. volvulus* larvae [47-49], and the *Litomosoides sigmodontis* murine model [50-54]. Co-administration of both of the *O. volvulus* antigens enhanced parasite killing as compared to single antigen immunizations, with all of the adjuvants inducing Th2 responses. Antigen specific IgG1 was the dominant antibody isotype that developed in protected immunized mice. Based on chemokine levels within the diffusion chambers, it appears that eosinophils, macrophages and neutrophils participate in the killing mechanism. These findings suggest that the mechanism of protective immunity induced by the two *O. volvulus* antigens is multifactorial with roles for cytokines, chemokines, antibody and specific effector cells [55]. This observation was confirmed in the *B. malayi*–gerbil model, where it was demonstrated that serum

from gerbils immunized with the two *B. malayi* antigens on alum, killed the parasites *in vitro*, in collaboration with peritoneal exudate cells [46].

Thus, based on the two model systems, *O. volvulus* in mice and *B. malayi* in gerbils, an effective two-antigen vaccine against *O. volvulus* has been identified. It consists of the proteins *Ov*-103 and *Ov*-RAL-2, administered with an adjuvant that induces Th2 responses. Immunization with both antigens enhanced the protective immune response and the mechanism of protective immunity appears to be antibody and effector cell dependent, in both model systems.

As mentioned above a third small animal model, the *L. sigmodontis*-BALB/c mouse model, has been developed and used for studying anti-filarial immunity and vaccines [56, 57]. This model also allows full development of the infective larvae to adult worms producing circulating microfilariae. It will be worthwhile to incorporate this third model into future efficacy pipeline studies and validate the *L. sigmodontis* homologous of the *O. volvulus* vaccine candidates also in this filarial infection model in mice.

The need for a rational and efficient process to generate a robust pipeline of second generation vaccine candidate antigens

The disappointing results obtained many times during human proof of concept clinical trials, continue to highlight the challenges and limitations of how to best predict whether a vaccine candidate translates successfully from animal testing into humans [58, 59].

Many articles call for a change in paradigm from an empirical development strategy to a rational vaccine design [60-62]. Amongst the parameters driving decisions during the development of new vaccine targets, the current consensus is that antigen selection and optimization represents the foundation in vaccine design. In addition, it is essential to have available appropriate preclinical models, but it is also crucial to have optimal vaccine formulations, adjuvants and delivery strategies. These are essential elements to target the appropriate immune mechanisms of protection [63]. This is especially important when developing vaccines for infectious diseases, such as for onchocerciasis, because unfortunately scientific advances and tools are still trailing and there is also a need for safety and efficacy studies to be done more quickly, with more certainty and at lower costs.

For example, strategies to identify the ideal *Onchocerca* vaccine candidate antigens can rely on selection processes based on the knowledge of candidates inducing effective immune responses, identifying antibody-based epitopes via computational prediction tools, down-selection of candidates based on predictions of sequences that could induce immunopathology or allergy, and continuous assessment of parasite molecules by structural biology and stability assessments. Hence, systems biology approaches continue to lead the efforts seeking better understanding of the mechanisms of protection and safety of vaccines [61].

Considerable efforts have also been done in the area of novel adjuvant development. Subunit vaccines need help with secondary molecules modulating the immune responses. TOVA Initiative is also incorporating into the development path the evaluation of other adjuvants besides the traditional phosphate or hydroxide salts of aluminum such as oil-in-water emulsions and synthetic toll-like receptor agonists [62]. The objective is to select adjuvants that facilitate the most effective response, while in parallel investigate their optimized use, route and molecular mechanism.

Selecting and evaluating the ideal delivery route and system also provides a benefit towards rational vaccine design. Investigating the mechanisms to overcome pre-existing immunity, an understanding of the basis for the stimulation of memory responses, and examining the interface between innate and adaptive immunity can also maximize the potential for vaccines to trigger long-lasting immunity and protection.

Using 'omics to catalyze and accelerate the decision process for the discovery of second generation vaccine candidate antigens

Recent technology advancements of the 21st Century have allowed now the use of new animal or computer-based predictive models, biomarkers for safety and efficacy, and clinical evaluation techniques to assist in the improvement of predictability and efficacy needed along the critical path to move discoveries from the laboratory bench to licensure. Ultimately, developing and identifying methods to establish correlate markers or surrogate endpoints for protection will be necessary and essential [60].

The current accumulation of molecular data and expansion of filarial parasite RNA and DNA databases, as well as proteomic datasets, has already provided a fresh start by permitting a more rational approach to vaccine candidate discovery [64]. For instance, the availability of genomes for *B. malayi*, *L. sigmodontis* and *O. ochengi* has facilitated numerous secretome studies across the parasite lifecycle [65-67]. One group of vaccine candidates that was identified by this unbiased, high-throughput approach was a ShK toxin domain family in which each individual member contains six ShK domains; a situation that is unique to filarial nematodes [30]. These abundant secreted proteins probably have an immunomodulatory role [66, 68] that could be targeted using antigens incorporating rational mutation of critical amino acid residue(s); an approach that has been used successfully with CPI-2 [56, 69]. In addition, the *O. volvulus* genome, as well as the transcriptome and proteome of each stage from the definitive host (L3, molting L3, L4, adult male, adult female, and nodule and skin microfilaria stages), has been published recently [70, 71]. These new datasets, when combined with immunomics [72-76], have provided an opportunity to identify the antigens that, either alone or in combination, function as targets of natural acquired immunity against filariae. Recombinant protein or synthetic peptide arrays can be used to interrogate the genome-wide proteome of infectious pathogens consisting of the entire potential antigens using only small amounts of individual sera samples. This approach permits investigators to perform extensive longitudinal, epidemiological and surveillance analyses, as well as identifying immune responses at various stages of infections in the human host in a fashion not possible with other technologies [77, 78].

Using the immunomics approach with sera samples from putatively immune individuals from Cameroon and the Americas *versus* sera from infected individuals, six new potential vaccine antigens were identified. This was accomplished by screening for IgG1, IgG3 and IgE antibody responses against a protein array containing 362 *O. volvulus* recombinant proteins [71], and identifying those with a significant IgG1 and/or IgG3 reactivity with little-to-no IgE reactivity. Notably, four of these antigens (OVOC10819, OVOC5395, OVOC11598 and OVOC12235) are highly expressed during the development of the early stages of the infective stage larvae, L3, in the human host; these would be worthy candidates for testing their efficacy in a preventative vaccine model of infection. Interestingly, the two other proteins (OVOC8619 and OVOC7083) are highly expressed by the microfilariae and were mostly recognized by sera from the putatively immune individuals who never developed a patent infection with microfilaridermia; these would be worthy to be tested as vaccine candidates for a therapeutic vaccine [71].

The initial objective for the *Onchocerca* vaccine was to identify candidate antigens for a prophylactic vaccine to be administered to children under the age of five who have not yet had access to MDA with ivermectin (Table 1); the first generation of our vaccine candidates fulfilled this objective. However, the immunomics approach now opens new possibilities for also developing a safe anti-transmission or therapeutic vaccine. The immunomics studies reported by Bennuru et al. [71] were the first time in which the *O. volvulus* stage-specific genome-wide expression data was used to discover empirically

406 novel vaccine candidates. It would be of great interest to test the novel vaccine
407 candidates identified by the immunomics approach [71] in the *O. volvulus* diffusion
408 chamber mouse model [45] and *B. malayi* – gerbil infection model to validate whether
409 the immunomics approach actually have identified vaccine candidates that protect
410 against L3 and/or microfilariae.
411
412 Other potential applications of immunomic approaches include unbiased
413 characterization of the immune response at the site of infection. In the *O. ochengi*
414 system in cattle, a recent secretome analysis of nodule fluid identified almost 500 host
415 proteins that 'bathe' the adult worms *in vivo* [67]. Interestingly, these proteins were
416 dominated by antimicrobial proteins, such as cathelicidins, which probably originate
417 from the neutrophils that dominate the intranodular environment. A parallel approach
418 could be used to explore the immunological changes that occur within nodules in
419 animals displaying partial protection induced by vaccination. Such studies will be very
420 valuable in the future for the machine learning approach described below.
421
422 **Prospective: The potential for machine learning to accelerate the evaluation and**
423 **selection of vaccine candidates**
424
425 Decades of research on prototype anti-filarial vaccines in animal models, the application
426 of transgenic knockout mouse strains, and immunological studies of onchocerciasis
427 patients presenting different clinical phenotypes, has led to a broad consensus on the
428 characteristics of protective immunity and some of the key factors that drive

immunopathology. Thus, a Th2-biased immune response directed against incoming infective larvae, with a secondary (but important) role for a Th1 component and the modulating influence of T-regulatory cells, is associated with ‘benign’ protection [57, 79, 80]. Conversely, at least in humans, unregulated Th2 responses against microfilariae in conjunction with Th17-driven inflammation and profound eosinophilia lead to effective parasite killing, but at the price of a hyperreactive form of onchocerciasis exhibiting severe skin inflammation also called sowda if the inflammation is unilaterally predominant [81, 82]. This very rare condition is associated with certain genetic polymorphisms in immune-related genes [83, 84]. However, adverse reactions with a clear immunological component are possible in a wider range of patients, as is not uncommon with antifilarial chemotherapy [85, 86]. Consequently, accurately predicting whether a vaccine candidate is likely to be both safe and effective is very challenging using conventional approaches alone, especially as we lack animal models that recapitulate the pathology seen in human onchocerciasis.

Traditional statistical approaches can be powerful at disentangling these immunological events, but tend not to generalize well from model systems to humans. However, machine learning techniques have been developed to improve generalizability by tuning models to maximize prediction accuracy to independent test samples, and tend to deal with large numbers of variables better than traditional statistical approaches [87, 88]. Such methods have been used successfully to analyze immune responses to bacterial infection using whole blood transcriptional signatures [89], and to detect local pathogen-specific immune profiles in peritoneal dialysis patients [87]. In principle, by combining

vaccinology read-outs from animal models and natural immunity in humans, it may therefore be possible to improve the selection of vaccine candidates earlier than currently possible. Thus, by identifying robust markers of immunity that generalize well, such approaches may help bridge the divide between development, preclinical, and clinical phases of vaccine development (Fig. 2).

Concluding remarks

Although it was previously considered that *O. volvulus* infections can be controlled using only MDA with ivermectin, it is becoming increasingly clear that without additional modalities such as drugs which kill or permanently sterilize the adult worms and/or a vaccine, elimination of onchocerciasis from Sub Saharan Africa may remain an unfulfilled goal. Vaccines aimed at preventing infection (anti-L3), and/or reduce microfilariae in adults and children with onchocerciasis could be the essential complement for the successful control or elimination of both diseases.

The successful vaccines developed against taeniases and the major advances already made in development of human anthelminthic vaccines [90], show that it is indeed possible to develop and test protective vaccines against multicellular parasites. In regard to *O. volvulus*, the human studies have suggested that protective immunity can develop in humans. The experimental and natural infections of calves have demonstrated that protective immunity does develop and that vaccines can protect animals from infection under natural conditions. Moreover, using the small animal

models for antigen screening have already accomplished the identification of two lead vaccine candidates; now the challenge is to optimize and formulate these vaccines for human usage, which can take advantage of the procedures currently being developed for the human hookworm and schistosome vaccines [91, 92], making the process potently quicker than usually expected (see Outstanding Questions). Efforts to develop novel diagnostic assay that support the monitoring of current and future control measures are underway and are expected to also provide in the near future diagnostic assays that can predict efficacy of the prophylactic and therapeutic vaccines in human clinical trials.

The task ahead is to assure continued pre-clinical development by convincing potential donors that *O. volvulus* vaccine production and testing is a realistic goal worth supporting. The potential development of drug resistance to the drugs used for MDA and the many years of MDA now being anticipated to control onchocerciasis might provide such impetus.

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Figure Legends:

Figure 1: Schematics that illustrates the down-selection process that resulted in the selection of the two most promising vaccine antigens for future clinical development.

Figure 2: Combining a systems analysis of response to vaccines and machine learning algorithms to help predict vaccine efficacy. (A) Applying machine learning to experimental infections across multiple model systems and species can help identify which immune variables throughout the time course of an infection most reliably predict infection load, while ensuring the trained models generalize well across biological systems. (B) These optimized models may then be useful in predicting vaccine efficacy in human trials in two ways: identifying what data to collect and predicting likely vaccine efficacy using incomplete data that are typical of human field studies.

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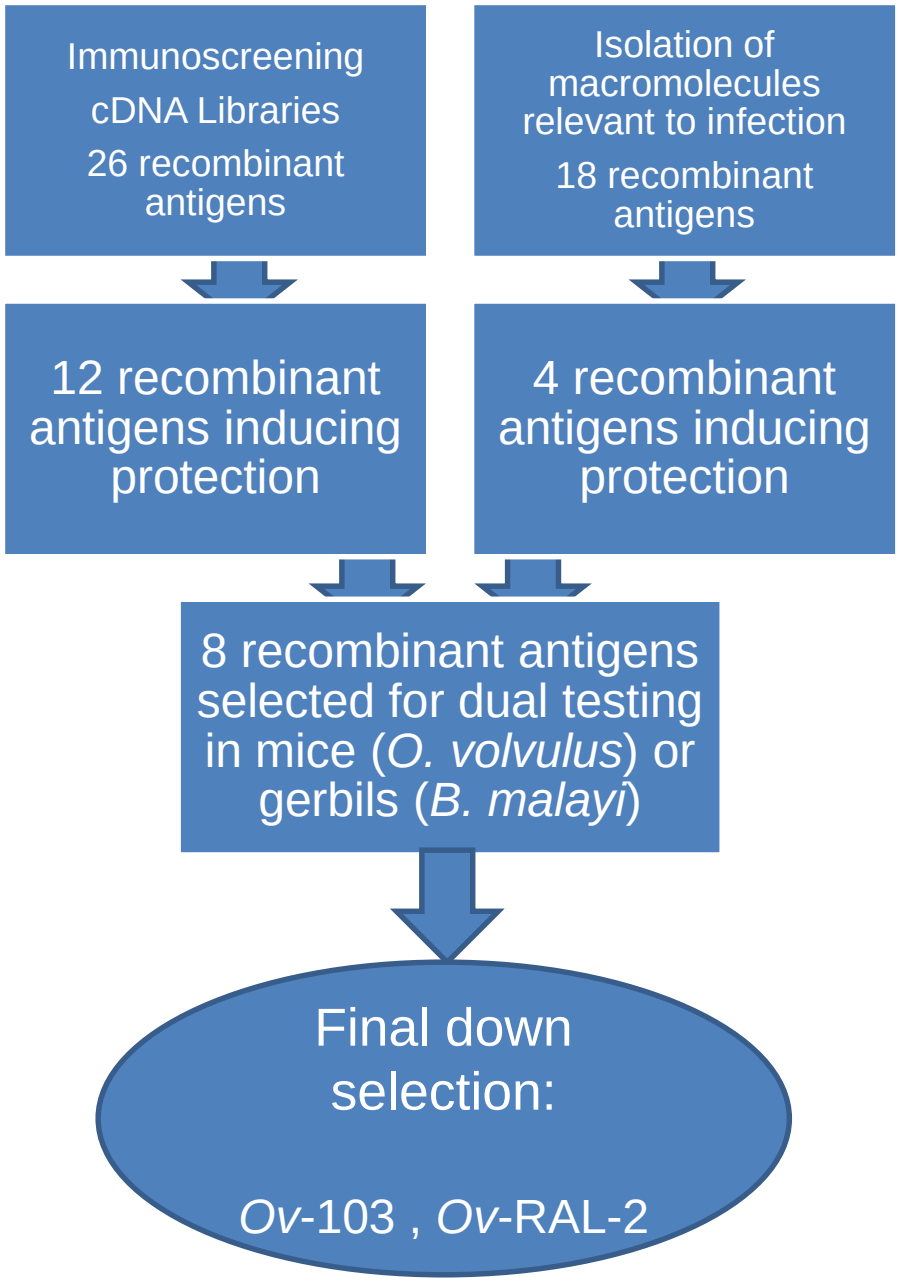
Figure 1


Immunoscreening
cDNA Libraries
26 recombinant antigens
Isolation of macromolecules relevant to infection
18 recombinant antigens
12 recombinant antigens inducing protection
4 recombinant antigens inducing protection
8 recombinant antigens selected for dual testing in mice (O. volvulus) or gerbils (B. malayi)
Final down selection:
Ov-103 , Ov-RAL-2

Figure

Click here to download Figure Figure 2.tiff

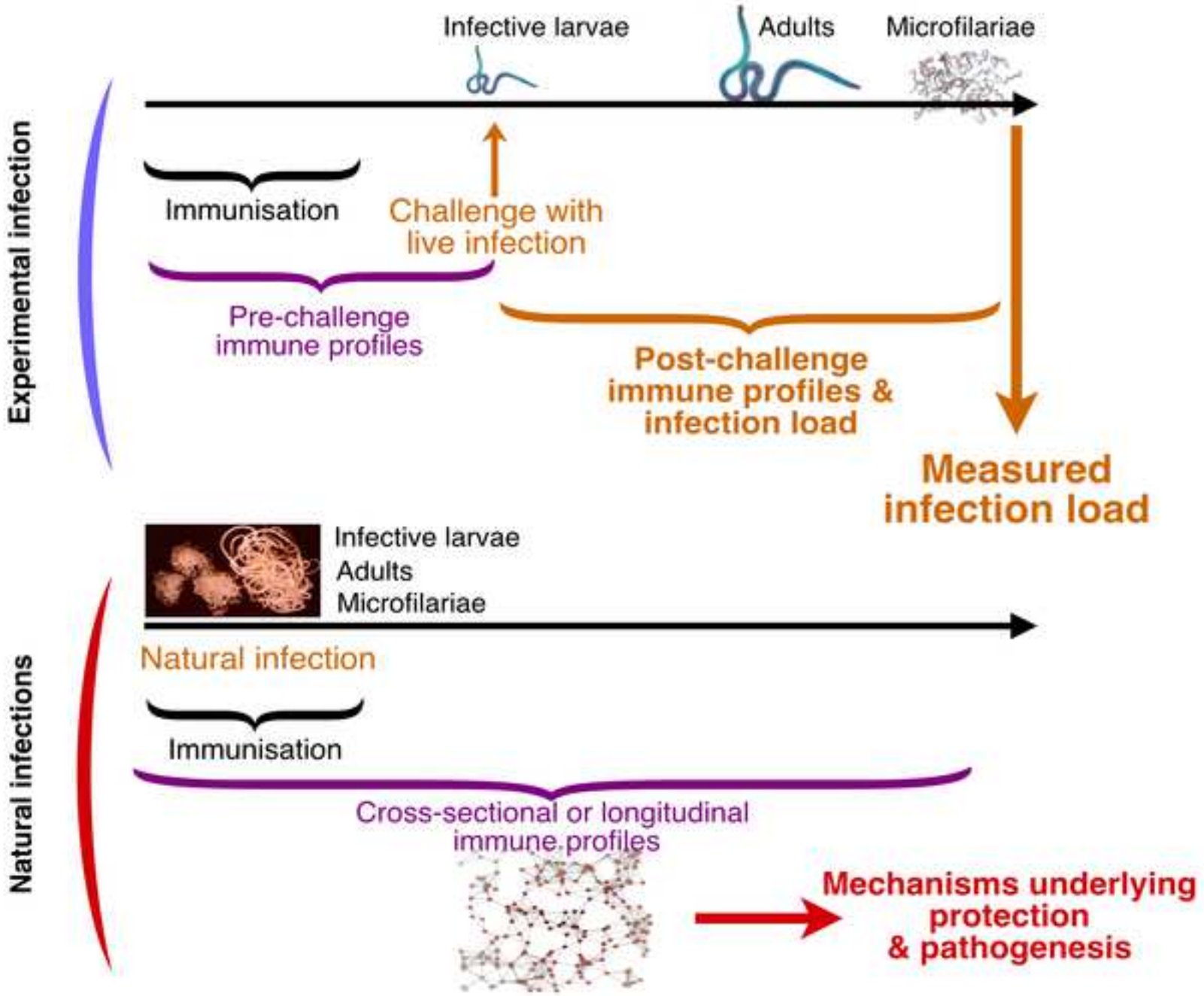


Table 1: Target product profiles for prophylactic and therapeutic onchocerciasis vaccines

Characteristic	Desired target – prophylactic[a]	Desired target – therapeutic (if different)[b]
Indication	A vaccine to protect against infection with infective larvae and to reduce adult worm burden and microfiladermia for the purpose of reducing morbidity and transmission	A vaccine to reduce microfiladermia for the purpose of reducing morbidity and transmission
Target population	Children <5 years	older children and adults that already carry adult worms
Route of administration	Intramuscular injection	
Product presentation	Single-dose vials; <0.5 ml volume of delivery	
Dosage schedule	Maximum of 3 immunizations given 4 weeks apart	
Warnings and precautions/pregnancy and lactation	Mild to moderate local injection site reactions such as erythema, oedema and pain, the character, frequency, and severity of which is similar to licensed recombinant protein vaccines. Less than 0.01% risk of urticaria and other systemic allergic reactions. Incidence of serious adverse reactions no more than licensed comparator vaccines	
Expected efficacy	>50% efficacy at preventing establishment of incoming worms; >90% reduction of microfilariae (based on current animal model results)	>99% reduction of microfilariae
Co-administration	All doses may be co-administered and/or used with other infant immunization programmes	
Shelf life	4 Years	
Storage	Refrigeration between 2 to 8 degrees Celsius. Cannot be frozen. Can be out of refrigeration (at temperatures up to 25 degrees) for up to 72 hours	
Product registration	Licensure by the Food and Drug Administration and/or the European Medicine Agency	
Target price	Less than $10 per dose for use in low- and middle-income countries.	

[a]adapted from [2].

[b], the assumptions for the blank cells are similar to those expected for the prophylactic vaccine

Box 1: Key points that support the advancement and progress towards an onchocerciasis vaccine

- It remains unlikely that onchocerciasis can be eliminated entirely through ivermectin mass treatments
- An international consortium launched in 2015 a new global initiative, known as TOVA – The Onchocerciasis Vaccine for Africa – with the goal of evaluating and pursuing vaccine development as a complementary control tool
- A rational design for the antigen discovery and selection process before embarking into advanced vaccine development of the onchocerciasis vaccine resulted in the identification of two recombinant proteins – Ov-103 and Ov-RAL-2 – that individually or in combination induced significant protection against infection

Outstanding Questions

- What additional tools are needed to support the elimination of onchocerciasis in Africa?
- Adjuvants are an important component for vaccine delivery; additional adjuvants that may increase efficacy should be tested versus alum formulated vaccines
- The need to optimize the *O. volvulus* vaccine in regard to dosage, number of immunization and ability to provide sufficient memory
- Should we proceed to identify new vaccine candidates for prophylactic and/or therapeutic vaccines using more rational approaches?
- How can new technologies and artificial intelligence can catalyze and accelerate the evaluation and selection of more effective vaccine candidates leading to a greater chance of their translation into safe and efficacious human vaccines?
- The development of diagnostic assays that can predict efficacy of the prophylactic and therapeutic vaccines in human clinical trials

Author Supplementary Material Figure 1

Author Supplementary Material Figure 1 legend

TREPAR-D-17-00158

Responses to the reviewers' critiques and editorial comments:

Reviewer 1:

This is an interesting and timely article on Onchocerca vaccines from a team that is at the cutting edge of this endeavour. The ms is reasonably well balanced but contains information that is a distraction to the main message, and lacks information on areas that I feel are critical for a balanced article. Specific comments are as follows.

1. A figure outlining the different approaches to antigen discovery would be useful [COMMENT FROM THE EDITOR –Good suggestion from the reviewer. I would say that this figure would be more relevant than the current figure 1]

Response: We decided to add a supplement table that outlines exactly how each vaccine protein was identified; hopefully that will fulfill the gap identified in Figure 1.

2. The long list of antigens is too much like a laundry list and would be best captured as a table of vaccine antigens and their methods of discovery, percent efficacy in animal models, protective properties, adjuvants, etc. [COMMENT FROM THE EDITOR – Another good suggestion from the reviewer that should translate into an easier, more pleasant reading experience]

Response: We have added a Supplement Table 1 that has the characteristics of all the 15 antigens initially identified as potential vaccine candidates. Subsequently, we hope that ready the text is less of a laundry list and the reader can find more information on each protein with the relevant details on methods of discovery, percent efficacy in animal models, protective properties, adjuvants, etc. as suggested.

3. On page 8, ranking of antigens is described; where is this information provided? It would be helpful for readers to see how ranking is done.

Response: we appreciate the comment and consequently added a new text in the review that explain the ranking and the corresponding supplement table l that depicts the score given to each vaccine antigen.

4. On page 8 there is brief mention of Th2 responses being required for protection. This is interesting and would benefit from expansion. Has vaccination in any filarial nematode been looked at in a setting where Th2 immunity is blocked, either genetically (Th2 deficient mice, eosinophil deficient mice?) or via neutralizing antibodies against Th2 cytokines, etc?

Response: We thank the reviewer for raising this question. Indeed, the requirement for Th2 responses for adaptive immunity (naturally developing or vaccine-mediated) is consistent with evidence from immunity to *O. volvulus* in mice and other filarial models, and specifically, *L. sigmodontis* in which a full patent infection is possible in wild type BALB/c mice. A sentence with corresponding references has been added according.

5. Some researchers believe that irradiated helminths will yield better protection than subunit vaccines, and there are already irradiated worm vaccines on the market for livestock filairiids (lungworm). Some discussion about irradiated flilarial parasites as vaccines is warranted. Has it

been done in an animal model for Oncho? Is (should) it be considered a gold standard in Oncho? Could sera from animals vaccinated with irradiated Oncho (or other filariids) be used to screen libraries or protein arrays? Can such an approach be feasibly scaled up for human use?

Response: The reviewer rightly points out that irradiated infective larvae are an effective means to immunize against filarial infections. This has indeed been used in laboratory settings, mostly, and typically achieves around 70% protection, which has been shown in the *O. volvulus* and *Litomosoides sigmodontis* models to be mediated by the killing of larvae after infection. However, this approach cannot scale to veterinary or human vaccines (the lungworm *Dictyocaulus viviparus* is not, however, a filariid). A sentence has been added to the ms to address this.

6. On page 12, the authors talk about 'filaria unique 6-ShK toxins domain family". They are Shk by definition (not a filariid) and they are found in many other species of nematodes. Can the author explain what they mean here?

7. Two lines on from the ShK story the authors mention "rational ablation" and reference the term to a citation. Please explain herein what this actually means.

Response to 6 and 7: an expansion on these family members of vaccine candidates in filaria was added including new references.

<u>Text now read:</u> One group of vaccine candidates that was identified by this unbiased, high-throughput approach was a ShK toxin domain family in which each individual member contains six ShK domains; a situation that is unique to filarial nematodes [30]. These abundant secreted proteins probably have an immunomodulatory role [62, 64] that could be targeted using antigens incorporating rational mutation of critical amino acid residue(s); an approach that has been used successfully with CPI-2 [65, 66].

8. How many of your animal model-selected antigens matched those that obtained significant protection based on the proteome array studies when probed with endemic normal human subjects?

Response: Good question. Interestingly, Ov103, Ov-RAL-2 and Ov-CPI-2 were part of the top 25 immunoreactive proteins recognized by the PI sera using the protein arrays. However, as in our recent publication we focused on the identification of potential novel vaccine candidates, we have selected to show just the six (OVOC10819, OVOC5395, OVOC11598, OVOC12235, OVOC8619, and OVOC7083) based on their IgG1 and/or IgG3 reactivity (with little to no IgE reactivity), and subsequently discuss them in this review as 2nd generation of vaccine candidates.

9. Page 14, para 1 (commencing "It is important to note...". This para is out of place here and should be moved elsewhere or deleted. [COMMENT FROM THE EDITOR – Could be moved to the concluding remarks section or see last comment by reviewer 2]

Response: this paragraph was moved to the section “Evaluation and selection of the best vaccine candidates for a prophylactic vaccine using two small animal models” as suggested by Reviewer 2.

10. The text from the bottom of page 16 through to the top of page 18 is somewhat off topic and too esoteric given the focus of the remainder of the article. I think this should be removed and

replaced with more information on the development of resistance in humans (and animal models), including a focus on what is known about endemic normals and how they can guide antigen discovery and inform protective MoA. [COMMENT FROM THE EDITOR – I like the forward looking aspect of this section, but perhaps it can be turned into a more succinct text box on AI/machine learning, to leave room to address the reviewers suggestion]

Response: we agree with the editor's suggestion that this section should be shortened. We have reduced it to roughly a third of its original length, and removed the more technical explanations which the reader can now find in the references. We are not sure that this will fit into a text box; but it is up to the Editor to decide.

11. The human infectious diseases field in general is moving towards human challenge models. eg. hookworm, malaria, and now the development (unpublished) of a human challenge model for schistosomiasis (male worms only). Is this feasible for Oncho? Some discussion around this would be good. Even if not feasible, discussion around how vaccine trials will assess efficacy in the field would be helpful. Has modelling been done for Oncho vaccines with and without chemotherapy? [COMMENT FROM THE EDITOR – Good points to address in the outstanding questions/concluding remarks]

Response: For the cow model, we believe that the force of infection in Cameroon is sufficiently high so as not to require a challenge model. Specifically, we think it's feasible to obtain both safety and efficacy signal immunizing cattle and looking at outcomes in a natural field setting. For humans, we believe a similar situation may be in play. We could obtain an efficacy signal by conducting vaccine studies in an endemic setting. To develop a challenge model and working out dose and routes (subcutaneous vs intradermal vs intramuscular) would require years of investigative work and may not be on the critical path. A minor change was made in the text, hope this will be satisfactory.

Reviewer 2:

The review of Onchocerca volvulus vaccines by Lustigman and colleagues is an interesting overview of current data. I have the following comments which may further add to their review:

* In the abstract, the authors state that resistance is probably developing - I think the authors need to be more definitive in this statement, is there evidence for drug resistance?

Response: agree. We removed potential in the abstract and added in the text a new reference that describe recent genome-wide analyses which revealed genetic variation that significantly differentiated *O. volvulus* parasites that are good responders to treatment with ivermectin vs. sub-optimal responder (Doyle, SR et al 2017).

* Several of the sentences are particularly long, in some cases comprising an entire paragraph, making it difficult to take in all the information. If possible, these should be adapted into shorter sentences.

Response: We have attempted to shorten some of the sentences as requested.

* Page 3 - the sentence 'Such estimates indicate that onchocerciasis may not be eliminated for decades using current approaches' requires a full stop.

Response: done; thanks.

* Page 4 - can the author provide further information as to what classifies a protective immune response in terms of onchocerciasis. [COMMENT FROM THE EDITOR – Good suggestion from the reviewer to improve accessibility of the manuscript to the non-expert reader]

Response: description of the anti-L3 protective immunity within the *O. volvulus* endemic population and in the mouse model was added to the text.

* Under the 'Evaluation and selection section..' - page 8 - the authors say that 15 proteins were evaluated from previous studies, yet the figure implies that 16 were used for further evaluation.

Response: sorry about this confusion; one protein was cloned in 2005 by immunoscreening in addition to the 15 already identified in older previous studies, thus making the total 16. A sentence was added in the text to specify this.

* Page 8 the authors talk about the co administration of both antigens, but at this point in the paragraph it is not clear whether the O. volvulus antigens are being discussed or the B. malayi antigens or both.

Response: This issue has been clarified in the text

* Page 8 - Remove the full stop between Escherichia and coli.

Response: Done

* The sentence beginning 'Recent technology advancements of the 21st Century… (page 11)' needs amending - the technology advances are not considering the use of new models and techniques; they have allowed the development and use of new tools.

Response: thanks, the sentence was amended

* Page 13 - one of the objectives of the development of an Onchocerca vaccine is the potential use in children under the age of 5 that have not had access to MDA. Can the authors comment on the potential pitfalls/difficulties in developing a multi-use vaccine, in the sense of children and adults, particularly in relation to the adjuvant used to ensure the induction of a protective immune response? COMMENT FROM THE EDITOR – Excellent suggestion!]

Response: we added a text that explain that the prophylactic vaccine for children is based on a mathematical modelling (Turner H et a; 2015), while the one for the therapeutic vaccine with a potential use in young and adults is still hypothetical as we will have to prove first that it will be safe to target microfilariae immunologically.

* The authors have included Table 1 that details the target product profiles for potential Onchocerca vaccines. What is this information based on? Has any mathematical modelling shown that for a vaccine to be effective in the current situation, that there should be a >50% reduction in worm establishment and a >90% reduction of microfilariae? How feasible is this with current candidate antigens? In short, what is this vaccine trying to achieve and against which lifecycle stage - an anti L3 vaccine? The authors also mention that the vaccine could treat adult and children with onchocerciasis - in what way? [COMMENT FROM THE EDITOR – If you find it helpful to address this comment, an additional column can be added to the table to cite relevant references]

Response: we added a text that explain that the prophylactic vaccine in the TPP is based on a mathematical modelling (Turner H et a; 2015), while the one for the therapeutic vaccine is still hypothetical as we will have to prove first that it will be safe to target microfilariae immunologically.

* The authors review several studies that have identified various antigens of interest for vaccine trials, some which have been tested in animal models. The authors however, do not show how all these different antigens are related. Are the different methods of selection identifying similar antigens? Are they are all from the same lifecycle stage and if so what impact would that have on a potential vaccine? [COMMENT FROM THE EDITOR – I think the table suggested by reviewer 1 would also be helpful addressing this comment] Adjuvants are an important component for vaccine delivery - have any studies reported different efficacy levels in different adjuvants? [COMMENT FROM THE EDITOR – Perhaps another point to add to outstanding questions/concluding remarks]

Response: As suggested by reviewer #1 and the Editor, we added a table (Supplement 1) that show all the 15 *O. volvulus* vaccine antigens, how they were identified and which 8 were further selected based on scoring for extensive efficacy testing using the two animal models.

We added another point to Outstanding Questions that addressed the need to compare efficacy of the vaccine when formulated with other adjuvants

* Have any studies been carried out investigating dosage schedules for vaccine delivery? Do 3 doses given 4 weeks apart provide sufficient memory responses?

Response: Limited dosage studies have been performed at this time and it is unknown the duration of the memory response. In fact these two areas will be the focus of future research. This point was added to Outstanding Questions.

* Details of the Litomosoides sigmodontis-BALB/c mouse model could be moved up to the other animal model section.

Response: this paragraph was moved to the section "Evaluation and selection of the best vaccine candidates for a prophylactic vaccine using two small animal models" as suggested.

Editorial Comments

Line 51: Please add a sentence explaining how it is transmitted. I would also recommend adding a figure detailing the life cycle of the parasite.
Response: a sentence was added regarding the transmission,

I also referred to a supplement figure 1 that has the life cycle of O. volvulus taken for the CDC website. I added in a separated file the figure legend for this supplement figure alos taken from the same CDC website. Hope this is what you had in mind.

Line 78: For non-specialist readers it should be explained what loiasis is and why co-endemicity is a problem.
Response: a sentence to the reason why infection with the non-pathological Loa infection is a problem during MDA against Lymphatic filariasis and onchocerciasis was added.

Line 165: You may want to add a brief conclusion here and a segue into the next section.
Response: done, thanks.

Line 174: diffusion chambers, please explain briefly what these are.
Response: a sentence explaining the way diffusion cambers are used was added.

Line 357: It would be interesting to know the symptoms/manifestations of this severe form of the disease.
Response: a sentence explaining sowda clinical manifestation was added.

Line 455: The outstanding questions box lists interesting, broad questions. I think our readers would also like to know your opinion on which specific 2-3 questions should be addressed in the next 5 years and how. You can add those to the outstanding questions box and discuss them further in the concluding remarks.
Response: Thanks or this comment. We added three new outstanding questions as listed below as well as edited the conclusion section as needed.

- **Adjuvants are an important component for vaccine delivery; additional adjuvants that may increase efficacy should be tested versus alum formulated vaccines**
- **The need to optimize the *O. volvulus* vaccine in regard to dosage, number of immunization and ability to provide sufficient memory**
- **The development of diagnostic assays that can predict efficacy of the prophylactic and therapeutic vaccines in human clinical trials**

References: I would be grateful if you could:
1. Italicize all genus and species names in the reference list - **Done**
2. Provide full details for all references (30 and 36 seem to be incomplete; I was unable to find reference 7)
Response: thanks for noting these gaps in the full citations

Reference 7 was updated, it is now reference 14

14. Sher, A. (1988) Vaccination against parasites: special problems imposed by the adaptation of parasitic organisms to the host immune response. In The Biology of Parasitism, eds. P.T. Englund & A. Sher, pp. 169-82. New York:, Alan R. Liss, Inc.

Reference 30 was updated: it is now reference 66

66. Armstrong, S.D. et al. (2014) Comparative Analysis of the Secretome from a Model Filarial Nematode (Litomosoides sigmodontis) reveals Maximal Diversity in Gravid Female Parasites. Mol Cell Proteomics. 13(10):2527-44

Reference 36 was updated: it is now reference 71

71. Bennuru, S. et al. (2016) Stage-Specific Transcriptome and Proteome Analyses of the Filarial Parasite Onchocerca volvulus and Its Wolbachia Endosymbiont. MBio 7 (6). pii: e02028-16.

Table 1: Please include the table in the revised version of the main manuscript.

When cells are left blank, what does that mean? Please clarify using a footnote.

Response: A footnote was added saying that the assumptions for the blank cells are similar to those in the column of prophylactic vaccine

Was the full table adapted from this publication or only this column? Please clarify in a reply to this comment.

Response: many thanks for noticing this; you are right, only the column of prophylactic vaccine was adapted and the column on therapeutic vaccine is new.

Outstanding Questions

- What additional tools are needed to support the elimination of onchocerciasis in Africa?
- Adjuvants are an important component for vaccine delivery; additional adjuvants that may increase efficacy should be tested versus alum formulated vaccines
- The need to optimize the *O. volvulus* vaccine in regard to dosage, number of immunization and ability to provide sufficient memory
- Should we proceed to identify new vaccine candidates for prophylactic and/or therapeutic vaccines using more rational approaches?
- How can new technologies and artificial intelligence can catalyze and accelerate the evaluation and selection of more effective vaccine candidates leading to a greater chance of their translation into safe and efficacious human vaccines?
- The development of diagnostic assays that can predict efficacy of the prophylactic and therapeutic vaccines in human clinical trials